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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
JANICE BETH YEVED JING	RICHMAN-EISENSTAT YU	Winnipeg, Manitoba, Canada Winnipeg, Manitoba, Canada

☐ *Additional inventors are being named on the ____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

MODULATION OF MESENCHYMAL CELLS

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☒ Customer Number 1059

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☒ Firm or Individual Name	BERESKIN & PARR				
Address	40 King Street West				
Address					
City	Toronto	State	Ontario	ZIP	M5H 3Y2
Country	Canada	Telephone	416-364-7311	Fax	416-361-1398

ENCLOSED APPLICATION PARTS *(check all that apply)*

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☒ Drawing(s) *Number of Sheets* 6 ☐ Other (specify)

☒ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☒ A check or money order is enclosed to cover the filing fees

FILING FEE AMOUNT ($)

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 022095 $80.00

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: ____.

Respectfully submitted,

SIGNATURE M. Gravelle Date AUG. 1, 2002

TYPED or PRINTED NAME MICHELINE GRAVELLE REGISTRATION NO. *(if appropriate)* 40,261

TELEPHONE 416-364-7311 Docket Number: 9157-27

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Complete if Known	
Application Number	
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First Named Inventor	JANICE BETH YEVED RICHMAN-EISENSTAT
Examiner Name	
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METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card #4535 ☐ Money Order ☐ Other ☐ None

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	740	201	370	Utility filing fee	
106	330	206	165	Design filing fee	
107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below		Fee Paid
Total Claims	[ ] − 20** =	[ ]	X [ ]	=	0.00
Independent Claims	[ ] − 3** =	[ ]	X [ ]	=	0.00
Multiple Dependent			[ ]	=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claim, if not paid
109	84	209	42	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0.00

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

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105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3)** ($) 0.00

SUBMITTED BY				Complete *(if applicable)*	
Name (Print/Type)	Micheline Gravelle	*Registration No (Attorney/Agent)*	40,261	*Telephone*	(416) 364-7311
Signature	M. Gravelle			*Date*	AUGUST 1, 2002

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Patent Application Data Sheet

Application Information

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Suggested Group Art Unit::	
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Applicant Information

Inventor Authority Type::	Inventor
Primary Citizenship Country::	Canada
Status::	Full Capacity
Given Name::	JANICE
Middle Name::	BETH YEVED
Family Name::	RICHMAN-EISENSTAT
Name Suffix::	
City of Residence::	Winnipeg
State or Prov. Of Residence::	Manitoba
Country of Residence::	CANADA
Street of mailing address::	195 Montrose St.
City of mailing address::	Winnipeg
State or Province of mailing address::	Manitoba
Country of mailing address::	CANADA
Postal or Zip Code of	

[redacted]

mailing address::	R3M 3L9
Inventor Authority Type::	Inventor
Primary Citizenship Country::	China
Status::	Full Capacity
Given Name::	JING
Middle Name::	
Family Name::	YU
Name Suffix::	
City of Residence::	Winnipeg
State or Prov. Of Residence::	Manitoba
Country of Residence::	CANADA
Street of mailing address::	798 McDermot Ave., Suite #2
City of mailing address::	Winnipeg
State or Province of mailing address::	Manitoba
Country of mailing address::	CANADA
Postal or Zip Code of mailing address::	R3E 0T5

Correspondence Information

Correspondence Customer Number::	001059
Phone Number::	(416) 364-7311
Fax Number::	(416) 361-1398

[redacted]

E-Mail Address:: mgravelle@bereskinparr.com

Representative Information

Representative Customer Number:: 001059

Domestic Priority Information

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Foreign Priority Applications

Country::	Application Number::	Filing Date::	Priority Claimed

Assignee Information

Assignee name::

Street of mailing address::

City of mailing address::

State or Province of mailing address::

Country of mailing address::

Postal or Zip Code of mailing address::

B&P File No. 9157-27

BERESKIN & PARR

UNITED STATES PROVISIONAL

Title: MODULATION OF MESENCHYMAL CELLS

Inventors: JANICE BETH YEVED RICHMAN-EISENSTAT and JING YU

B&P File No. 9157-27

TITLE: MODULATION OF MESENCHYMAL CELLS

FIELD OF THE INVENTION

The invention relates to methods of modulating intracellular calcium signalling in mesenchymal cells (such as synovial fibroblasts), methods of treating arthritis and methods of drug delivery to mesenchymal cells, as well as methods to diagnose IgA-receptor-mediated mesenchymal inflammation.

BACKGROUND OF THE INVENTION

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a common chronic inflammatory and autoimmune disorder of unknown etiology that attacks adults and children (Choy and Panayi, 2001). Patients with RA have a poor long-term prognosis, with 80% becoming disabled after 20 years (Scott et al, 1987). Current treatments do not improve this prognosis. As in the population worldwide, the prevalence of RA in Manitobans is 1.3% and has been steadily rising (Peschken et al 1998). Insights into the cell biology of this disorder will go far to developing improved treatments that prevent disability and improve long-term prognosis.

RA is characterized by synovial inflammation, proliferation and progressive joint destruction (reviewed in Jenkins et al, 2002). The immune reaction begins in the synovial lining of the joint, with lymphocytes playing a significant role in acute disease and a lesser role in chronic disease. The earliest pathologic changes in the disease are microvascular injury and increased vascular permeability, accompanied by an influx of inflammatory cells (CD4 lymphocytes, neutrophils, and plasma cells) in the perivascular space. Cytokines, lymphokines, and chemokines are released. TNFα, IL-1 and IL-6 are the key cytokines that drive inflammation in RA. Patients develop swelling, pain, and joint stiffness with the onset of vascular injury and angiogenesis in the synovial membrane. Synovial proliferation and the evolving inflammation exacerbate these symptoms and progressively limit joint motion. Neutrophils

accumulate in the synovial fluid in response to local production of IL-8. B lymphocytes mature into plasma cells which locally produce rheumatoid factor and other antibodies that further aggravate the inflammation. Immune complexes activate the complement system, releasing chemokines and increasing vascular permeability. Immune complexes also promote phagocytosis, leading to greater lysosomal enzyme release and the digestion of collagen, cartilage matrix, and elastic tissues. The release of oxygen free radicals injures cells, which release phospholipids that fuel the arachidonic acid cascade and exacerbate the local inflammatory response. Proliferating synovium forms an invasive pannus, eroding through cartilage and subchondral bone. Of the two types of synoviocytes, type A monocyte-like and type B fibroblast-like, the type B fibroblast-like cells stimulate the cartilage and bone destruction of chronic disease. Chondrocytes release their own proteases and collagenases, and further contribute to this self-perpetuating local immune response.

The initial inciting factor and the precise mechanism of these complex cellular interactions remain unknown. However, it is clear that RA is characterized by increased activity of the pro-inflammatory transcription factor, NFκB, in synovial fibroblasts. NFκB stimulates production of cytokines and adhesion molecules, including TNF-α, IL-1β, IL-6, IL-8 and ICAM-1.

Patients with RA may also develop systemic vasculitis, neurologic, pulmonary, cardiac and/or liver abnormalities. The number and severity of the extra-articular features vary with the duration and severity of disease. Extra-articular complications are seen in patients with high titers of rheumatoid factor (RF). RF is an immunoglobulin that binds other immunoglobulins at their Fc components, forming immune complexes. RF may consist of IgM, IgA, IgE and/or IgG isotypes, and may be found in several other diseases including Sjogren's syndrome, subacute bacterial endocarditis, mixed cryoglobulinemia, systemic lupus erythematosis, scleroderma, sarcoidosis, idiopathic pulmonary fibrosis and malignancies. However, the combined elevation of IgM-RF and IgA-RF is highly specific for RA and is very rarely found in rheumatic diseases other than RA (Jonsson et al, 1998). In a cross-

sectional study, the majority (74%) of RA patients had elevations of 2-3 RF isotypes, and 67% had the combined elevation of IgA and IgM (Jonsson and Valdimarsson, 1992). Of those patients with RA, 65% are positive for IgA-RF and 92% are positive for IgM-RF (Gioud-Paquet et al, 1987).

IgA-RF can occur in serum and synovial fluid, and is predominantly polymeric (Otten et al, 1991; Schrohenloher et al, 1986). Several studies have reported significant clinical implications to IgA-RF in RA. RA patients with a predominant increase in IgA-RF have more erosive disease (Jorgensen et al, 1996. IgA-RF is associated with extra-articular manifestations of RA (Jonsson et al, 1995; Pai et al, 1998). Detection of IgA-RF early in disease predicts poorer prognosis with a more rapidly progressive course (Teitsson et al, 1984; Pai et al, 1998; Houssien et al, 1997).

Polymeric Immunoglobulin Receptor

IgA exists in different isoforms (Mestecky et al, 1999). B lymphocytes residing in submucosal tissues produce similar proportions of polymeric IgA1 and IgA2 subclasses, secreting at least two IgA molecules linked together by a J chain. Epithelial cells of the respiratory and gastrointestinal tracts abundantly express the polymeric immunoglobulin receptor (pIgR) which serves to transcytose polymeric IgA from the submucosa (the basolateral surface of the epithelium) to the lumenal (apical) surface. At the apical surface, proteolytic cleavage of the pIgR releases secretory component (SC) bound to dIgA into mucosal secretions, called secretory IgA (sIgA). SC stabilizes sIgA from proteolytic degradation by bacterial enzymes and helps neutralize pathogens, especially viruses. sIgA in mucosal secretions is the first line of defense, acting to bind microorganisms and thereby limiting adhesion and colonization. IgA may neutralize viruses and bacterial toxins by binding to antigenic determinants important in the microorganism's interaction with cellular receptors. Additional roles for sIgA are postulated to include transport of immune complexes out through the epithelial surface by the pIgR.

In contrast to mucosal secretions where sIgA prevails, the predominant form of IgA in human serum is monomeric IgA (mIgA) from B lymphocytes in the bone marrow and spleen. While the pIgR will selectively mediate transport

of polymeric IgA across epithelial cells, this receptor does not bind monomeric IgA. IgA present in secretions therefore differs in biochemical properties from IgA found in serum. The polymerization state and the presence of SC might be expected to result in unique effector functions for different forms of IgA depending on the site of production and intended point of action.

Mucosal epithelial cells of the airway and intestine abundantly express the pIgR which functions to transfer its ligand, polymeric IgA (pIgA), from the blood and submucosal B lymphocytes (basolateral surface) to the lumenal (apical) surface and into external secretions (Mostov et al, 1995). IgA is one of the first lines of host immune defense in mucosal secretions. The lung is the second greatest site of dIgA transport, exceeded only by the intestine. Total IgA transport is roughly 5-15 gms per day in an adult human with 15% transported into airway secretions, so pIgR transcytosis is clearly a significant pathway (Childers et al, 1989). In fact, IgA represents about 5-10% of the total protein in bronchoalveolar lavage fluid (Bell et al, 1981). The pIgR and bound ligands are very rapidly endocytosed from the basolateral surface of the epithelial cells, delivered to endosomes, and eventually transcytosed via vesicles to the apical surface of the epithelial cell. At the apical surface, the extracellular, ligand-binding domain of the pIgR is proteolytically cleaved and released together with its ligand into external secretions. This cleaved fragment of the pIgR is known as secretory component (SC). SC bound to polymeric IgA is known as secretory IgA (sIgA), and stabilizes IgA against proteolytic degradation by bacterial enzymes.

Cleavage of the pIgR at the apical surface is not extremely rapid, so there is a pool of uncleaved pIgR at the apical surface. Most of the apically endocytosed ligand recycles back to the apical surface. In contrast, only 20% of pIgR at the basolateral surface recycles back to the basolateral surface. The predominant route is for 80% of pIgR to traffic from the basolateral to the apical surface. These trafficking events occur regardless of whether pIgR is bound to its ligand, dIgA. The molecular determinants of these protein trafficking events are encoded in the 103 amino acid cytoplasmic domain of pIgR (Mostov et al, 1995). This domain contains highly conserved signals for

intracellular sorting and transcytosis, including signals for rapid endocytosis and for avoiding degradation in lysosomes. Upon binding of pIgR to pIgA, phospholipase C is activated and phosphokinase C stimulate apical delivery, IP3 releases calcium and calmodulin sequesters the basolateral retrieval signal. Gsα, cAMP and protein kinase A stimulate apical delivery of pIgR (Mostov and Kaetzel, 1999). The trafficking events of pIgR across epithelial cells are clearly under very tight control.

pIgA binds to the pIgR on mucosal epithelial cells via the J chain. In addition, pIgA, which is heavily glycosylated, can bind to asialoglycoprotein receptors on liver cells. In contrast, binding to white blood cells (neutrophils, eosinophils, monocytes/macrophages) occurs by attachment of the Fc portion of IgA to FcαR (also known as CD89) expressed on these cells (Kerr and Woof, 1999; Morton et al, 1996).

Fc-Alpha Receptors for IgA

Binding to white blood cells (neutrophils, eosinophils, monocytes/macrophages) occurs by attachment of the Fc portion of IgA to Fc-alpha receptors (FcαR; also known as CD89) expressed on these cells (Kerr and Woof, 1999; Morton et al, 1996). Neutrophils and monocytes/macrophages constitutively express FcαR as a 55-75 kd protein, while eosinophils express FcαR as a 70-100 kd protein with increased glycosylation (Albrechtsen M, et al, 1988; Monteiro et al, 1990). FcαR expression on monocytes and neutrophils increases in response to TNF-α, IL-1, GM-CSF, LPS or phorbol esters; IFN-γ and TGF-β1 decrease expression (discussed in Deo et al, 1998). The gene for FcαR is located on chromosome 19 and encodes several alternatively spliced isoforms of the receptor's α-chain (55-110 kD; Morton et al, 1996). FcαR can trigger release of inflammatory mediators and phagocytosis of IgA-coated particles (Yeaman and Kerr, 1987; Patry et el, 1995). IgA-coated neutrophils and macrophages phagocytose particles, bacteria and immune complexes more efficiently than uncoated cells. Although the concentration of the predominantly monomeric IgA in blood is high enough to completely saturate the thousands of FcαR on

neutrophils, mIgA will not trigger signal transduction in PMNs unless the receptors are crosslinked (Stewart et al., 1994). The pIgA and sIgA have the potential to crosslink FcαR on cell surfaces due to their polymeric composition. So, during times of infection when submucosal B cells are stimulated to increase production of specific pIgA, myeloid cells recruited to sites of inflammation are better prepared for their functions in the mucosal lumen. FcαR-induced calcium release and subsequent cytokine production depend on association with the FcR γ-chain (Morton et al, 1995). In vivo studies in transgenic mice show that while FcR γ chain is important for FcαR-triggered phagocytosis, CR3 (CD11b/CD18) is required for FcαR-mediated antibody-dependent cellular cytotoxicity (van Egmond et al, 1999).

FcαR may play a role in cancer in addition to its function against microbial pathogens: IgA antitumor antibodies or bispecific antibodies directed to FcαR and tumor antigens effectively lyse tumor cells (Deo et al, 1998). Deo's work and that of others highlight FcαR as a potential immunotherapeutic target of malignant and infectious diseases (Valerius et al, 1997; van de Winkel et al, 1997). The novel finding of the FcαR on synovial fibroblast thus indicates that targeting this receptor would be a promising and novel therapeutic approach for inflammatory diseases, such as arthritis.

IgA and Calcium signaling

Sustained intracellular calcium concentrations are associated with cell proliferation in response to several growth factors. The cell signaling pathway for several growth factors (i.e. PDGF, EGF, IGF and FGF) is almost identical to that of ligand binding to the pIgR on epithelial cells. In general, most of the growth factors induce tyrosine kinase activity upon binding to their cognate receptor, i.e. phosphorylate cytoplasmic proteins on tyrosine residues. These receptor proteins relay signals via receptor dimerization and activation of the tyrosine kinase domain. The receptor-ligand complex internalizes and induces a number of cytoplasmic changes, including increases in intracellular cAMP levels, degradation of phosphoinositides to inositol phosphates and DAG. These latter two products effect the release of calcium ions from intracellular

stores and the activation of protein kinase C (PKC). PKC coordinates further protein modification leading to the regulation of the transcription of genes. *A potential growth factor effect of pIgA has never been investigated.*

SUMMARY OF THE INVENTION

The inventors have unexpectedly found that the polymeric immunoglobulin receptor (pIgR) and the Fc alpha receptor (FcαR) is expressed on synovial fibroblasts from patients with rheumatoid arthritis (RA) and osteoarthritis (OA). The inventors have also shown that synovial tissue from arthritis patients express pIgR. Furthermore, the inventors have shown that incubating RA synovial fibroblasts with IgA causes an increase in the proliferation of the fibroblasts. As a result, inhibiting signalling through IgA receptors may be an effective means of treating arthritis and other inflammatory diseases.

Accordingly, the present invention provides a method of modulating the proliferation of a mesenchymal cell comprising administering to a cell or animal in need thereof an effective amount of an agent that can modulate an IgA receptor on a mesenchymal cell.

In one embodiment, the present invention provides a method of inhibiting the proliferation of a mesenchymal cell comprising administering to a cell or animal in need thereof an effective amount of an IgA receptor antagonist.

In a further embodiment, the present inventioin provides a method of treating an inflammatory condition caused by IgA binding to an IgA receptor on a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a patient in need thereof.

In one embodiment, the present invention provides a method of treating a patient with arthritis comprising administering an effective amount of an IgA receptor antagonist to a patient in need thereof.

The binding of IgA to an IgA receptor is known to induce intracellular calcium signalling and cause a number of calcium dependent effects. Accordingly, the present invention also provides a method of modulating intracellular calcium signalling in a mesenchymal cell comprising

administering to a cell or animal in need thereof an effective amount of an agent that can modulate an IgA receptor on a mesenchymal cell. In one embodiment, the present invention provides a method of inhiting intracellular calcium signalling in a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a cell or animal in need thereof.

The discovery of IgA receptors on mesenchymal cells allows the development of methods to target delivery of a compound or substance to a mesenchymal cell. Accordingly, the present invention also includes a method of delivering a substance to a mesenchymal cell comprising administering an effective amount of a conjugate comprising the substance coupled to an IgA receptor ligand to an animal or cell in need thereof.

The discovery of IgA receptors on synovial fibroblasts allows development of diagnostic assays to detect IgA receptor immediated diseases or inflammatory conditions including arthritis (including rheumatoid arthritis, osteoarthritis, spondyloarthropathies), as well as other inflammatory diseases such as Crohn's disease, Ulcerative colitis, Behcet's disease, Sjogren's disease and vasculitides.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 shows immunofluorescence staining for pIgR and FcαR in primary cell cultures of both RA and OA synovial fibroblasts.

Figure 2 shows RT-PCR product bands for IgA-binding domain of FcαR and the cytoplasmic domain of pIgR in both RA and OA synovial fibroblasts.

Figure 3 shows immunohistochemical staining of pIgR in human RA and OA synovial tissue samples.

Figure 4 shows that mIgA increases proliferation of RA synovial fibroblasts treated with mIgA compared to serum-free media alone. In addition, pIgA in 1% serum increases in RA synovial fibroblast proliferation compared to 1% serum alone.

Figure 5 shows a dose-dependent increase in NFκB activity in both RA and OA synovial fibroblasts treated with increasing concentrations of pIgA.

Figure 6 shows a western blot confirming that the different scFv clones recognize purified J-chain protein.

DETAILED DESCRIPTION OF THE INVENTION

I. Therapeutic Methods

As mentioned above, the present inventors have determined that IgA receptors, including pIgR and Fc-alpha R, are present on RA and OA synovial fibroblasts as well as synovial tissue from arthritis patients and that binding the receptor causes the proliferation of synovial fibroblast cells. Therefore the present invention includes all diagnostic and therapeutic methods for treating conditions that are mediated through modulation of signalling through IgA receptors on mesenchymal cells.

Broadly stated, the present invention provides a method of modulating the proliferation of a mesenchymal cell comprising administering to a cell or animal in need thereof an effective amount of an agent that can modulate an IgA receptor on a mesenchymal cell.

The term "modulate" as used herein includes the inhibition or suppression of a function or activity as well as the induction or enhancement of a function or activity and interference with the interaction between any isoform of IgA and its receptor such as pIgR or FcαR. For example, an agent that can modulate IgA receptors includes agents that can inhibit or block the

signalling through this receptor (receptor antagonists) as well as agents that can induce or stimulate signalling through the receptor (receptor agonists).

The term "IgA receptor" means any receptor on a mesenchymal cell (such as a synovial fibroblast) that can bind an isoform of IgA. The receptor may also bind other immunoglobulins. In a preferred embodiment, the IgA receptor on the mesenchymal cell is pIgR or FcαR.

The term "pIgR" as used herein denotes a polymeric immunoglobulin receptor and means a receptor on cells that binds polymeric IgA (pIgA), dimeric IgA (dIgA) and polymeric IgM (pIgM) but not monomeric forms of IgA. The term includes the pIgR that has been previously described on epithelial cells (Piskurich et al., *J. Immuno.* 154:1735-1747, 1995) as well as any analogs, homologues, derivatives or variants (including splice variants) of the known pIgR molecules.

The term "FcαR" as used herein denotes the Fc-alpha receptor, also known as CD89, and means a receptor on cells that binds any isoform of IgA by its Fc portion. The term includes the FcαR that has been previously described on white blood cells (Morton et al, *Crit. Rev. Immunol.* 16: 423-440, 1996) as well as any analogs, homologues, derivatives or variants (including splice variants) of the known FcαR molecules.

The term "mesenchymal cell" as used herein includes fibroblasts, synovial cells, smooth muscle cells and endothelial cells. The mesenchymal cell will express pIgR or a pIgR-like protein and/or the Fc-alpha receptor. The mesenchymal cell is preferably a synovial fibroblast cell.

The term "a cell" as used herein includes a single cell as well as a plurality or population of cells. Administering an agent to a cell includes both *in vitro* and *in vivo* administrations.

The term "animal" as used herein includes all members of the animal kingdom, including humans. Preferably, the animal to be treated is a human.

The term "effective amount" as used herein means an amount effective, at dosages and for periods of time necessary to achieve the desired result, e.g. to modulate cell proliferation.

The IgA receptor antagonist (such as a pIgR or FcαR antagonist) can be any agent that inhibits signalling through the IgA receptor and results in an inhibition of function caused by signalling through the receptor including an inhibition of cell proliferation or an inhibition of pIgR- or FcαR-mediated endocytosis. In one embodiment, the IgA receptor antagonist will inhibit the binding of pIgA to pIgR or FcαR on mesenchymal cells. The IgA receptor antagonist may be an antibody that binds, but does not activate the pIgR or FcαR on mesenchymal cells, preferably synovial fibroblasts cells, and results in an inhibition of the binding of IgA with the resultant inhibition of cell proliferation. Examples of other pIgR or FcαR antagonists are provided in Section II.

In one embodiment, the present invention provides a method of preventing or inhibiting the proliferation of a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a cell or animal in need thereof.

The term "preventing or inhibiting the proliferation of a mesenchymal cell" means that the proliferation of the mesenchymal cell in the presence of the IgA receptor antagonist is decreased as compared to the level of proliferation in the absence of the antagonist. Proliferation of mesenchymal cells can be measured using a variety of techniques known in the art including the techniques as described in Example 1.

The methods of the invention can be used to treat any condition wherein it is desirable to modulate IgA receptor activity on mesenchymal cells. Such conditions include, but are not limited to, inflammatory diseases including arthritides (including rheumatoid arthritis, osteoarthritis, spondyloarthropathies), Crohn's disease, ulcerative colitis, Behcet's disease, Sjogren's disease and vasculitides.

Accordingly, the present invention provides a method of treating an inflammatory condition caused by IgA binding to an IgA receptor on a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a patient in need thereof.

As used herein, and as well understood in the art, "treating" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treating" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The inventors have shown that pIgA stimulates NF-κB activity in RA synovial fibroblasts. As RA is characterized by increased NF-κB activity inhibiting the activity of this pro-inflammatory transcription factor (by inhibiting an IgA receptor) may be useful in treating arthritis. Accordingly, the present invention provides a method of treating a patient with arthritis comprising administering an effective amount of an IgA receptor antagonist to a patient in need thereof.

As mentioned previously, the binding of IgA to an IgA receptor induces intracellular calcium signalling which further induces a variety of calcium dependent effects. Accordingly, the present invention provides a method of preventing or inhibiting intracellular calcium signalling in a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a cell or animal in need thereof.

The term "preventing or inhibiting intracellular calcium signalling" means that the intracellular level of calcium in a mesenchymal cell in the presence of the a IgA receptor antagonist is decreased as compared to the level of intracellular calcium in cells in the absence of the agent. Calcium levels can be measured using a variety of known techniques including using fluorescence spectrophotometric and imaging techniques.

Intracellular calcium signalling is important for several processes in cell biology, including cell division, cytokine/chemokine/growth factor production, cell movement and contraction. Therefore, inhibiting calcium signalling can

inhibit a variety of calcium dependent effects. Accordingly, the present invention provides a method of inhibiting the contraction of a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a cell or animal in need thereof.

The present invention further provides a method of inhibiting the production of inflammatory mediators or growth factors comprising administering an effective amount of an IgA receptor antagonist to a cell or animal in need thereof. In a preferred embodiment, the method inhibits the production of NF-κB.

II. Agents That Modulate pIgR or FcαR

The finding by the present inventors that pIgR or FcαR are on mesenchymal cells allows the discovery and development of agents that modulate pIgR or FcαR for use in modulating diseases mediated through an IgA receptor, such as pIgR or FcαR, on mesenchymal cells.

The present invention includes the use of any and all agents that modulate pIgR or FcαR in the methods of the invention. The agent can be any type of substance, including, but not limited to, nucleic acids (including antisense oligonucleotides, proteins (including antibodies), peptides, peptide mimetics, carbohydrates, organic compounds, inorganic compounds, small molecules, drugs, pIgR or FcαR ligands, soluble forms of pIgR or FcαR, pIgR or FcαR agonists, pIgR or FcαR antagonists, agents that inhibit pIgR or FcαR agonists, polymeric IgA (pIgA), dimeric IgA (dIgA) and polymeric IgM (pIgM) and fragments of these IgA or IgM molecules. Examples of some of the agents that modulate pIgR or FcαR are provided below.

(i) Antibodies

In one embodiment, the agent that can modulate pIgR is an antibody that binds to pIgR. Within the context of the present invention, antibodies are understood to include monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', $F(ab')_2$, scFv and Fv fragments) and recombinantly produced binding partners. Antibodies to pIgR may act as pIgR agonists or pIgR antagonists. For example, whole antibodies may act as pIgR agonists

by stimulating the receptor while antibody fragments may act as pIgR antagonists by blocking the ability of pIgR ligands (such as pIgA) to bind pIgR.

In one embodiment, the antibody is an antibody fragment that acts as a pIgR antagonist. In a specific embodiment, the antibody fragment is a single chain Fv antibody. Single chain-Fv fragments can be isolated from a phage display library. The preparation of scFv antibodies to pIgR is described in Example 2.

In one embodiment, the agent that can modulate FcαR is an antibody that binds to FcαR. Within the context of the present invention, antibodies are understood to include monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', $F(ab')_2$, scFv and Fv fragments) and recombinantly produced binding partners. Antibodies to FcαR may act as FcαR agonists or FcαR antagonists. For example, whole antibodies may act as FcαR agonists by stimulating the receptor while antibody fragments may act as FcαR antagonists by blocking the ability of FcαR ligands (such as mIgA or pIgA) to bind FcαR.

In one embodiment, the antibody is an antibody fragment that acts as a FcαR antagonist. In a specific embodiment, the antibody fragment is a single chain Fv antibody. Single chain-Fv fragments can be isolated from a phage display library. The preparation of scFv antibodies to FcαR is described in Example 2.

In another embodiment, the antibody is a pIgR agonist. Examples of antibodies that are pIgR agonists include pIgA and pIgM. Antibodies to pIgR may be prepared using techniques known in the art such as those described by Kohler and Milstein, Nature 256, 495 (1975) and in U.S. Patent Nos. RE 32,011; 4,902,614; 4,543,439; and 4,411,993, which are incorporated herein by reference. (See also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, and Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988, which are also incorporated herein by reference).

In another embodiment, the antibody is a FcαR agonist. Examples of antibodies that are FcαR agonists include mIgA and pIgA. Antibodies FcαR may be prepared using techniques known in the art such as those described by Kohler and Milstein, Nature 256, 495 (1975) and in U.S. Patent Nos. RE 32,011; 4,902,614; 4,543,439; and 4,411,993, which are incorporated herein by reference. (See also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, and Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988, which are also incorporated herein by reference).

(ii) Antisense oligonucleotides

In another embodiment, the agent that can modulate pIgR or FcαR is an antisense oligonucleotide that acts as a pIgR or FcαR antagonist, respectively, by inhibiting the expression of the pIgR or FcαR gene. The term "antisense oligonucleotide" as used herein means a nucleotide sequence that is complimentary to its target, e.g. the pIgR or FcαR gene. The sequence of the pIgR and FcαR genes are known in the art for many species, for example, see Piskurich et al., *J. Immunol.* 154:1735-1747, 1995, and Maliszewski et al, *J. Exp. Med.* 172:1665-1672, 1990.

The term "oligonucleotide" as used herein refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and intersugar (backbone) linkages. The term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted oligonucleotides may be preferred over naturally occurring forms because of properties such as enhanced cellular uptake, or increased stability in the presence of nucleases. The term also includes chimeric oligonucleotides which contain two or more chemically distinct regions. For example, chimeric oligonucleotides may contain at least one region of modified nucleotides that confer beneficial properties (e.g. increased nuclease

resistance, increased uptake into cells), or two or more oligonucleotides of the invention may be joined to form a chimeric oligonucleotide.

The antisense oligonucleotides of the present invention may be ribonucleic or deoxyribonucleic acids and may contain naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil. The oligonucleotides may also contain modified bases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thiolalkyl guanines, 8-hydroxyl guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines, adenines, or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

Other antisense oligonucleotides of the invention may contain modified phosphorous, oxygen heteroatoms in the phosphate backbone, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. For example, the antisense oligonucleotides may contain phosphorothioates, phosphotriesters, methyl phosphonates, and phosphorodithioates. In an embodiment of the invention there are phosphorothioate bonds links between the four to six 3'-terminus bases. In another embodiment phosphorothioate bonds link all the nucleotides.

The antisense oligonucleotides of the invention may also comprise nucleotide analogs that may be better suited as therapeutic or experimental reagents. An example of an oligonucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in the DNA (or RNA), is replaced with a polyamide backbone which is similar to that found in peptides (P.E. Nielsen, et al Science 1991, 254, 1497). PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives in vivo and in vitro. PNAs also bind stronger to a complimentary DNA sequence due to the lack of charge repulsion between the PNA strand and the DNA strand. Other oligonucleotides may contain

nucleotides containing polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may have morpholino backbone structures (U.S. Patent No. 5,034,506). Oligonucleotides may also contain groups such as reporter groups, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an antisense oligonucleotide. Antisense oligonucleotides may also have sugar mimetics.

The antisense nucleic acid molecules may be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. The antisense nucleic acid molecules of the invention or a fragment thereof, may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed with mRNA or the native gene e.g. phosphorothioate derivatives and acridine substituted nucleotides. The antisense sequences may be produced biologically using an expression vector introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense sequences are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the vector is introduced.

(iii) Peptide Mimetics

The present invention also includes peptide mimetics of the pIgR or FcαR proteins. Such peptides may include competitive inhibitors, enhancers, peptide mimetics, and the like. All of these peptides as well as molecules substantially homologous, complementary or otherwise functionally or structurally equivalent to these peptides may be used for purposes of the present invention.

"Peptide mimetics" are structures which serve as substitutes for peptides in interactions between molecules (See Morgan et al (1989), Ann. Reports Med. Chem. 24:243-252 for a review). Peptide mimetics include synthetic structures which may or may not contain amino acids and/or peptide bonds but retain the structural and functional features of a pIgR peptide, or

enhancer or inhibitor of the pIgR peptide. Peptide mimetics also include peptoids, oligopeptoids (Simon et al (1972) Proc. Natl. Acad, Sci USA 89:9367); and peptide libraries containing peptides of a designed length representing all possible sequences of amino acids corresponding to a peptide of the invention.

Peptide mimetics may be designed based on information obtained by systematic replacement of L-amino acids by D-amino acids, replacement of side chains with groups having different electronic properties, and by systematic replacement of peptide bonds with amide bond replacements. Local conformational constraints can also be introduced to determine conformational requirements for activity of a candidate peptide mimetic. The mimetics may include isosteric amide bonds, or D-amino acids to stabilize or promote reverse turn conformations and to help stabilize the molecule. Cyclic amino acid analogues may be used to constrain amino acid residues to particular conformational states. The mimetics can also include mimics of inhibitor peptide secondary structures. These structures can model the 3-dimensional orientation of amino acid residues into the known secondary conformations of proteins. Peptoids may also be used which are oligomers of N-substituted amino acids and can be used as motifs for the generation of chemically diverse libraries of novel molecules.

(iv) Other substances

In addition to antibodies and antisense oligonucleotides, other substances that can modulate pIgR or FcαR can also be identified and used in the methods of the invention. In one embodiment, the pIgR or FcαR modulator is a protein or peptide that can bind to pIgR or FcαR. The pIgR- or FcαR-binding peptides may be isolated by assaying a sample for peptides that bind to pIgR or FcαR. Any assay system or testing method that detects protein-protein interactions may be used including co-immunoprecipitation, crosslinking and co-purification through gradients or chromatographic columns may be used. Biological samples and commercially available libraries may be tested for pIgR- or FcαR-binding peptides. For example,

labelled pIgR or FcαR may be used to probe phage display libraries. In addition, antibodies that bind pIgR or FcαR may be used to isolate other peptides with pIgR or FcαR binding affinity. For example, labelled antibodies may be used to probe phage display libraries or biological samples. Additionally, a DNA sequence encoding a pIgR protein may be used to probe biological samples or libraries for nucleic acids that encode pIgR- or FcαR-binding proteins.

Substances which can bind pIgR or FcαR may be identified by reacting pIgR or FcαR, respectively, with a substance which potentially binds to pIgR or FcαR, then detecting if complexes between the respective receptor and the substance have formed. Substances that bind pIgR or FcαR in this assay can be further assessed to determine if they are useful in modulating or inhibiting pIgR or FcαR and useful in the therapeutic methods of the invention.

Accordingly, the present invention also includes a method of identifying substances which can bind to pIgR or FcαR comprising the steps of:

(a) reacting pIgR or FcαR and a test substance, under conditions which allow for formation of a complex between the pIgR or FcαR and the test substance, and

(b) assaying for complexes of pIgR or FcαR and the test substance, for free substance or for non complexed pIgR or FcαR, wherein the presence of complexes indicates that the test substance is capable of binding pIgR or FcαR.

Conditions which permit the formation of substance and IgA receptor complexes may be selected having regard to factors such as the nature and amounts of the substance and the protein.

The substance-IgA receptor complex, free substance or non-complexed proteins may be isolated by conventional isolation techniques, for example, salting out, chromatography, electrophoresis, gel filtration, fractionation, absorption, polyacrylamide gel electrophoresis, agglutination, or combinations thereof. To facilitate the assay of the components, antibody against pIgR or FcαR or the substance, or labelled pIgR or FcαR, or a

labelled substance may be utilized. The antibodies, pIgR or FcαR, or substances may be labelled with a detectable substance.

The pIgR or FcαR or the test substance used in the method of the invention may be insolubilized. For example, the pIgR or FcαR or substance may be bound to a suitable carrier. Examples of suitable carriers are agarose, cellulose, dextran, Sephadex, Sepharose, carboxymethyl cellulose polystyrene, filter paper, ion-exchange resin, plastic film, plastic tube, glass beads, silica, polyamine-methyl vinyl-ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, etc. The carrier may be in the shape of, for example, a tube, test plate, beads, disc, sphere etc.

The insolubilized pIgR or FcαR or substance may be prepared by reacting the material with a suitable insoluble carrier using known chemical or physical methods, for example, cyanogen bromide coupling.

The pIgR or FcαR or test substance may also be expressed on the surface of a mesenchymal cell in the above assay.

The pIgR or FcαR gene or protein may be used as a target for identifying lead compounds for drug developments. The invention therefore includes an assay system for determining the effect of a test compound or candidate drug on the activity of the pIgR or FcαR gene or protein.

Accordingly, the present invention provides a method for identifying a compound that modulates pIgR or FcαR activity comprising:

(a) incubating a test compound with pIgR or FcαR protein or a nucleic acid encoding the pIgR or FcαR protein; and

(b) determining the effect of the test compound on the pIgR or FcαR protein activity or pIgR or FcαR gene expression and comparing with a control (i.e. in the absence of a test compound) wherein a change in the pIgR or FcαR protein activity or pIgR or FcαR gene expression as compared to the control indicates that the test compound is a potential modulator of the pIgR or FcαR gene or protein.

In one embodiment, pIgR or FcαR activity may be assessed by measuring intracellular calcium levels as previously described.

III. Compositions

The present invention also includes pharmaceutical compositions containing the agents that can modulate or inhibit pIgR or FcαR for use in the methods of the invention. Accordingly, the present invention provides a pharmaceutical composition for modulating the proliferation of a mesenchymal cell comprising an effective amount of an agent that can modulate an IgA receptor in admixture with a suitable diluent or carrier. The present invention also includes a pharmaceutical composition for preventing or inhibiting the proliferation of a mesenchymal cell comprising an effective amount of an IgA receptor antagonist in admixture with a suitable diluent or carrier. The present invention further provides a pharmaceutical composition for preventing or treating arthritis comprising an effective amount of an IgA receptor antagonist in admixture with a suitable diluent or carrier. In a preferred embodiment, the IgA receptor antagonist is a pIgR or FcαR antagonist.

Such pharmaceutical compositions can be for intralesional, intravenous, topical, rectal, parenteral, local, inhalant or subcutaneous, intradermal, intramuscular, intrathecal, transperitoneal, oral, and intracerebral use. The composition can be in liquid, solid or semisolid form, for example pills, tablets, creams, gelatin capsules, capsules, suppositories, soft gelatin capsules, gels, membranes, tubelets, solutions or suspensions.

The pharmaceutical compositions of the invention can be intended for administration to humans or animals. Dosages to be administered depend on individual needs, on the desired effect and on the chosen route of administration.

The pharmaceutical compositions can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients, and such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985).

On this basis, the pharmaceutical compositions include, albeit not exclusively, the active compound or substance in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids. The pharmaceutical compositions may additionally contain other agents such as other agents that can modulate or inhibit cell proliferation or that are used in treating inflammatory conditions such as arthritis.

IV. Targeted Delivery

The finding by the present invention that pIgR and FcαR are on mesenchymal cells allows the development of methods to target the delivery of substances directly to mesenchymal cells. Accordingly, the present invention provides a method of delivering a substance to a mesenchymal cell comprising administering an effective amount of a conjugate comprising the substance coupled to an IgA receptor ligand to an animal or cell in need thereof.

The substance can be any substance that one wishes to deliver, including therapeutics and diagnostics, to a mesenchymal cell. In a specific embodiment, the substance is useful in treating an inflammatory condition such as arthritis.

The ligand can be any molecule that can bind the IgA receptor, including pIgA or pIgM, as well as the ligands described in Section II.

The substance may be coupled to the IgA receptor ligand either directly or indirectly. In direct coupling, the substance and ligand are physically linked such as by covalent binding or physical forces such as van der Waals or hydrophobic interactions. In indirect coupling, the substance and ligand are joined through another molecule or linker. As one example, the substance and ligand may be joined through a bispecific antibody that binds both the substance and linker.

Conjugates of the substance and the IgA receptor ligand may be prepared using techniques known in the art. There are numerous approaches for the conjugation or chemical crosslinking of proteins and one skilled in the art can determine which method is appropriate for the substance to be

conjugated. The method employed must be capable of joining the substance with the IgA receptor ligand without interfering with the ability of the ligand to bind to the IgA receptor and without significantly altering the activity of the substance. If the substance and ligand are both proteins, there are several hundred crosslinkers available in order to conjugate the substance with the ligand. (See for example "Chemistry of Protein Conjugation and Crosslinking". 1991, Shans Wong, CRC Press, Ann Arbor). The crosslinker is generally chosen based on the reactive functional groups available or inserted on the substance. In addition, if there are no reactive groups a photoactivatible crosslinker can be used. In certain instances, it may be desirable to include a spacer between the substance and the ligand. In one example, the ligand and substance may be conjugated by the introduction of a sulfhydryl group on the ligand and the introduction of a cross-linker containing a reactive thiol group on to the substance through carboxyl groups (Wawizynczak, E.J. and Thorpe, P.E. in Immunoconjugates: Antibody Conjugates in Radioimaging and Therapy of Cancer, C.W. Vogel (Ed.) Oxford University Press, 1987, pp. 28-55.; and Blair, A.H. and T.I. Ghose, J. Immunol. Methods 59:129 ,1983).

In another embodiment, the protein ligand and substance may be prepared as a fusion protein. Fusion proteins may be prepared using techniques known in the art. In such a case, a DNA molecule encoding the IgA receptor ligand is linked to a DNA molecule encoding the substance. The chimeric DNA construct, along with suitable regulatory elements can be cloned into an expression vector and expressed in a suitable host.

The conjugates of the invention may be tested for their ability to enter mesenchymal cells and provide the desired pharmacological effect using *in vitro* and *in vivo* models.

V. <u>Diagnostic Assays</u>

The finding by the present inventors that synovial fibroblasts and synovial tissue from arthritis patients have IgA receptors (such as pIgR and FcαR) allows the development of diagnostic assays to detect diseases mediated through IgA binding to an IgA receptor on a mesenchymal cell.

Such diagnostic assays can facilitate the development of tailored therapies for such diseases. Samples from patients can be obtained and tested for the presence of IgA receptors, such as pIgR or FcαR, on mesenchymal cells. The sample can be any sample that contains a mesenchymal cell including synovial fibroblasts and synovial tissue, connective tissue, endothelial cells and blood vessels, smooth muscle cells, or primary cell cultures of these cells derived from a tissue biopsy. Patients expressing an IgA receptor may be treated with IgA receptor antagonists as described above.

Accordingly, the present invention provides a method of detecting a condition associated with the activation of an IgA receptor on a mesenchymal cell comprising assaying a sample for (a) a nucleic acid molecule encoding an IgA receptor or a fragment thereof or (b) an IgA receptor or a fragment thereof. The IgA receptor is preferably pIgR or FcαR. In one embodiment, the condition associated with the activation of an IgA receptor on a mesenchymal cell is an inflammatory condition such as arthritides (including rheumatoid arthritis, osteoarthritis, spondyloarthropathies), Crohn's disease, ulcerative colitis, Behcet's disease, Sjogren's disease and vasculitides.

(i) Detecting Nucleic acid molecules encoding IgA receptors

Nucleotide probes can be prepared and used in the detection of nucleotide sequences encoding an IgA receptor or fragments thereof in samples, preferably pIgR or FcαR. The probes can be useful in detecting the presence of a condition associated with the activation of an IgA receptor on a mesenchymal cell or monitoring the progress of such conditions include inflammatory conditions including the arthritides (including rheumatoid arthritis, osteoarthritis, spondyloarthropathies), Crohn's disease, ulcerative colitis, Behcet's disease and Sjogren's disease and vasculitides. Accordingly, the present invention provides a method for detecting a nucleic acid molecule encoding an IgA receptor comprising contacting the sample with a nucleotide probe capable of hybridizing with the nucleic acid molecule to form a hybridization product, under conditions which permit the formation of the hybridization product, and assaying for the hybridization product.

The nucleotide probe may be labelled with a detectable substance such as a radioactive label which provides for an adequate signal and has sufficient half-life such as 32P, 3H, 14C or the like. Other detectable substances which may be used include antigens that are recognized by a specific labelled antibody, fluorescent compounds, enzymes, antibodies specific for a labelled antigen, and chemiluminescence. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleic acid to be detected and the amount of nucleic acid available for hybridization. Labelled probes may be hybridized to nucleic acids on solid supports such as nitrocellulose filters or nylon membranes as generally described in Sambrook et al, 1989, Molecular Cloning, A Laboratory Manual (2nd ed.).

Nucleic acid molecules encoding an IgA receptor can be selectively amplified in a sample using the polymerase chain reaction (PCR) methods and cDNA or genomic DNA. A nucleic acid can be amplified from cDNA or genomic DNA using oligonucleotide primers and standard PCR amplification techniques. The amplified nucleic acid can be cloned into an appropriate vector and characterized by DNA sequence analysis. cDNA may be prepared from mRNA, by isolating total cellular mRNA by a variety of techniques, for example, by using the guanidinium-thiocyanate extraction procedure of Chirgwin et al., Biochemistry, 18, 5294-5299 (1979). cDNA is then synthesized from the mRNA using reverse transcriptase (for example, Moloney MLV reverse transcriptase available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase available from Seikagaku America, Inc., St. Petersburg, FL).

(ii) Detecting IgA receptors

The presence of IgA receptors may be detected in a sample using IgA receptor ligands that bind to the IgA receptor. IgA receptor ligands are described above and include antibodies or other substances that can bind an IgA receptor. Accordingly, the present invention provides a method for detecting an IgA receptor comprising contacting the sample with a ligand that

binds to an IgA receptor which is capable of being detected after it becomes bound to the IgA receptor in the sample.

Ligands to an IgA receptor, such as antibodies specifically reactive with an IgA receptor, or derivatives thereof, such as enzyme conjugates or labeled derivatives, may be used to detect an IgA receptor in various biological materials. For example they may be used in any known immunoassays which rely on the binding interaction between an IgA receptor, and an antibody thereof. Examples of such assays are radioimmunoassays, enzyme immunoassays (e.g. ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination and histochemical tests. Thus, the antibodies may be used to detect and quantify an IgA receptor in a sample in order to determine its role in particular cellular events or pathological states, and to diagnose and treat such pathological states, such as arthritis.

Cytochemical techniques known in the art for localizing antigens using light and electron microscopy may be used to detect an IgA receptor. Generally, an antibody of the invention may be labelled with a detectable substance and an IgA receptor may be localised in tissue based upon the presence of the detectable substance.. Examples of detectable substances include various enzymes, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, biotin, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include radioactive iodine I-125, I-131 or 3-H. Antibodies may also be coupled to electron dense substances, such as ferritin or colloidal gold, which are readily visualised by electron microscopy.

Indirect methods may also be employed in which the primary antigen-antibody reaction is amplified by the introduction of a second antibody, having specificity for the antibody reactive against an IgA receptor. By way of example, if the antibody having specificity against an IgA receptor is a rabbit

IgG antibody, the second antibody may be goat anti-rabbit gamma-globulin labelled with a detectable substance as described herein.

Where a radioactive label is used as a detectable substance, an IgA receptor may be localized by autoradiography. The results of autoradiography may be quantitated by determining the density of particles in the autoradiographs by various optical methods, or by counting the grains.

The following non-limiting examples are illustrative of the present invention:

EXAMPLES

Example 1

Primary Cell Cultures of RA and OA Synovial Fibroblasts Express pIgR and FcαR.

(a) FcαR and pIgR protein expression was studied in RA and OA synovial fibroblasts by immunofluorescence. Both RA and OA cells showed staining for pIgR using either a rabbit (Dako) or goat (Sigma) antibody to SC and the appropriate FITC-conjugated secondary antibody. Figure 1 shows data obtained with the rabbit antibody to SC and a goat antibody to FcαR (representative experiment of n=4 different subjects with RA and n=3 for OA). Both RA and OA cells also showe staining for pIgR and for FcαR protein.

Primary cell cultures of RA and OA synovial fibroblasts express pIgR and FcαR mRNA by RT-PCR

(b) FcαR and pIgR mRNA expression was confirmed in both RA (n=3 different subjects) and OA (n=2) synovial fibroblasts by RT-PCR (Figure 2). Primers for the cytoplasmic domain of pIgR (sense: 5' GAC CCC ACT CCC TGC TCT AAC 3'; antisense: 5' AGA AGA GGG GAA GGA CGG GAG 3') and the IgA binding domain of FcαR (sense: 5' CCT CAG TCT GGG GCT TTC TTT 3'; antisense: 5' CTT GTT TGC GTC CAT GTG GTC 3') were used. The bands obtained from the RT-PCR product were DNA sequenced and confirmed to be partial sequences of their respective IgA receptors. These results confirm that RA and OA synovial fibroblasts express mRNA for pIgR and FcαR.

Synovial Tissue From Patients With Arthritis Express Receptors for IgA.

Acetone-fixed frozen sections of synovial tissue from arthritis patients undergoing joint replacement surgery were stained for IgA receptor expression. We have studied a total of 4 patients with RA and 3 other patients with OA using rabbit and goat antibodies to the SC portion of pIgR, and an HRP-conjugated secondary antibody (Jackson). Figure 3 shows a representative slide confirming the presence of pIgR in RA and OA synovial tissue. These results confirm that IgA receptor expression occurs in vivo in arthritis tissue as well as primary cell cultures of synovial fibroblasts.

IgA Stimulates Synovial Cell Proliferation.

RA is characterized by synovial cell proliferation. Primary cell cultures of RA synovial fibroblasts were grown in 96-well plates, serum starved for 1 day, and then treated with pIgA, mIgA, media with 1% serum or media without serum. Cell proliferation was assessed by BrdU ELISA. We found that mIgA slightly increases RA synovial cell proliferation compared to serum-free media alone. The cell proliferative effect 1% serum was slightly increased further with addition of pIgA (Figure 4). A proliferative effect has never been attributed to IgA.

IgA Stimulates NF-κb Activity in RA and OA Synovial Fibroblasts.

RA is characterized by increased activity of the pro-inflammatory transcription factor, NFκB, in synovial fibroblasts. Both RA and OA are chronic inflammatory conditions, but RA is an autoimmune inflammatory disease. To determine whether expression of IgA receptors might play a role in the inflammation of RA and OA, we asked whether pIgA stimulates NFκB activity in RA and OA synovial fibroblasts. We found that pIgA induced a dose-dependent increase in NFκB activity in both RA and OA synovial fibroblasts by DNA gel shift assay (Figure 5). This effect of increasing NFkB activity in synovial fibroblasts has never been described and has major implications for the role of IgA receptors in RA and OA.

These results implicate synovial pIgR and FcαR in the pathogenesis of RA, and possibly in OA.

Example 2

scFv selection methods and results:

A scFv phage library was reconstituted by pooling all first rounds of selection that the inventor had previously prepared. The scFv phage library that was originally used is described in: Sheets MD, Amersdorfer P, Finnern R, Sargent P, Lindquist E, Schier R, Hemingsen G, Wong C, Gerhart JC, Marks JD, Lindqvist E., Efficient construction of a large nonimmune phage antibody library: the production of high-affinity human single-chain antibodies to protein antigens. (*Proc Natl Acad Sci U S A.* 1998 May 26;95(11):6157-62.).

TG-1/pHen /phage$^{1st\ round}$ scFv. These selections were to the domain 6 of rat pIgR; and to cell selections for pIgR with MDCK cells transfected with rabbit pIgR and attempted in 12 different ways. These TG-1 from 13 tubes were combined and grown for isolating phage. These phage were used as the "reconstituted" phage library of scFv.

A. For selections against a mesenchymal FcαR:

1. Coat 3 immunotubes with mIgA (Biolynx; 6.5 λ/3 ml PBS) and block with 2% milk.
2. Preclear reconstituted phage library twice with 2 of the coated immunotubes.
3. Incubate SDS lysates from non-serum-starved mesenchymal cells (e.g. airway smooth muscle cells, ASM, purchased from Biowhittaker) with the 3rd coated tube (- to bind the putative ASM FcαR to the mIgA).
4. Incubate precleared phage with the 3rd tube.
5. Wash extensively (15-20) with PBS and elute the bound phage with 1% TEA (triethanolamine). Neutralize the high pH with 1M Tris pH 7.4.
6. Infect TG-1 E. coli with the phage, and grow.
7. Expand and rescue phage to repeat procedure 2 more times.
8. After round 3, randomly pick 96 colonies and screen these for scFv production (dot blot) and cell ELISAs using (a) U937 cells (myelomonocytic cell line that highly expresses the FcαR); and (b) ASM, both cell lines grown in a 96-well plate.

Results:

- 8 positives by ASM ELISA (OD450>0.2); 3 positives by U937 cell ELISA
  - BstN1 DNA digest of pcr products from the 3 clones showed unique patterns, suggesting isolation of 3 different scFv

B. For selections against a mesenchymal pIgR:

1. Coat 3 immunotubes with pIgA (10 λ myeloma serum/3 ml PBS) and block with 2% milk PBS.

2. Preclear reconstituted phage library twice with 2 of the coated immunotubes.

3. Incubate SDS lysates from non-serum-starved mesenchymal cells (e.g. ASM, purchased from Biowhittaker) with the 3rd coated tube (- to bind the putative ASM pIgR to the pIgA).

4. Incubate precleared phage with the 3rd tube.

5. Wash extensively (15-20) with PBS and elute the bound phage with 1% TEA. Neutralize with 1M Tris pH 7.4.

6. Infect TG-1 with the phage, and grow.

7. Expand and rescue phage to repeat procedure 2 more times.

8. After round 3, randomly pick 96 colonies and screen these for scFv production (dot blot) and cell ELISA using ASM and CALU-3 cells grown in a 96-well plate.

Results:

- 45 positives by ASM ELISA (includes 6 that were negative on CALU-3); 55 positives by CALU-3 ELISA.
  - Also screened by ELISA with human milk which contains secretory component, the extracellular part of pIgR, and found 26 positives (used OD450>0.4 with background reading of ~0.1); screened by ELISA with fetal calf serum-coated plate, and found 46 positives; rabbit anti-human SC antibody (Dako) used as positive control antibody
  - BstN1 DNA digest of pcr products from the all positive clones showed 12 unique patterns, suggesting isolation of 12 different scFv

C. For selections against J-chain:

Dr. Jiri Mestecky sent his PET32 plasmid containing the J-chain protein fused to thioredoxin and containing an IgA protease cleavage site and a 6His

tag for purification. This plasmid was infected into BL21 E. coli which were induced to produce the J-chain-thioredoxin fusion protein. This was purified by IMAC on a nickel resin.

1. Coat 2 immunotubes with thioredoxin (Sigma; 10 µg/ml) and block with 2% milk/PBS.
2. Coat 1 immunotube with the purified J-chain fusion protein, then block with 2%milk/PBS.
3. Preclear reconstituted phage library twice with the 2 thioredoxin-coated immunotubes.
4. Incubate precleared phage with the 3rd tube coated with the J-chain fusion protein.
5. Wash extensively (15-20) with PBS and elute the bound phage with TEA (triethanolamine). Neutralize the high pH with Tris buffer.
6. Infect [TG-1] E. coli with the phage, and grow.
7. Expand and rescue phage to repeat procedure 2 more times.
8. After round 3, randomly pick 96 colonies and screen these for scFv production (dot blot) and protein ELISA using one 96-well plate coated with thioredoxin and one plate coated with the J-chain fusion protein. (- To ensure that the scFv selected bind to J-chain and not to thioredoxin.)

Results:

- 30 positives by J-chain ELISA; none bound the thioredoxin-coated plate. (Background OD450 was ~0.07; chose OD450>0.2 to be positive.)
- 14 of these were induced to produce scFv (which contain a myc epitope tag) and all recognized J-chain by western blotting (mouse monoclonal anti-J-chain from InnoGenex was used as positive control); 9E10 (anti-myc mouse monoclonal antibody and anti-mouse HRP alone used as negative control). (Figure 6)
    - BstN1 DNA digest of pcr products from all positive clones showed 5 unique patterns, suggesting isolation of 5 different scFv.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood

that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

FULL CITATIONS FOR REFERENCES REFERRED TO IN THE SPECIFICATION

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WE CLAIM:

1. A method of modulating the proliferation of a mesenchymal cell comprising administering to a cell or animal in need thereof an effective amount of an agent that can modulate an IgA receptor on a mesenchymal cell.

2. A method of inhibiting the proliferation of a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a cell or animal in need thereof.

3. A method of treating an inflammatory condition caused by IgA binding to an IgA receptor on a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a cell or animal in need thereof.

4. A method according to claim 3 wherein the inflammatory condition is an arthritis.

5. A method according to claim 4 wherein the arthritides is selected from rheumatoid arthritis, osteoarthritis or a spondyloarthropathy.

6. A method according to claim 3 wherein the inflammatory condition is selected from Crohn's disease, ulcerative colitis, Behcet's disease, Sjogren's disease and a vasculitis.

7. A method of modulating intracellular calcium signalling in a mesenchymal cell comprising administering to a cell or animal in need thereof an effective amount of an agent that can modulate an IgA receptor on a mesenchymal cell.

8. A method of preventing or inhibiting intracellular calcium signalling in a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a cell or animal in need thereof.

9. A method of inhibiting the contraction of a mesenchymal cell comprising administering an effective amount of an IgA receptor antagonist to a cell or animal in need thereof.

10. A method of inhibiting the production of inflammatory mediators or growth factors comprising administering an effective amount of an IgA receptor antagonist to a mesenchymal cell or animal in need thereof.

11. A method according to any one of claims 1 to 10 wherein the IgA receptor is pIgR or FcαR.

12. A method according to any one of claims 2 to 11 wherein the IgA receptor antagonist inhibits the binding of pIgA to pIgR.

13. A method according to any one of claims 2 to 11 wherein the IgA receptor antagonist inhibits the binding of pIgA to FcαR.

14. A method according to any one of claims 2 to 13 wherein the IgA receptor antagonist is a scFv that binds pIgR or FcαR.

15. A method of identifying substances which can bind to pIgR on a mesenchymal cell comprising the steps of:

(a) reacting pIgR and a test substance, under conditions which allow for formation of a complex between the pIgR and the test substance, and

(b) assaying for complexes of pIgR and the test substance, for free substance or for non complexed pIgR, wherein the presence of complexes indicates that the test substance is capable of binding pIgR.

16. A method of identifying substances which can bind to FcαR on a mesenchymal cell comprising the steps of:

(a) reacting FcαR and a test substance, under conditions which allow for formation of a complex between the FcαR and the test substance, and

(b) assaying for complexes of FcαR and the test substance, for free substance or for non complexed FcαR, wherein the presence of complexes indicates that the test substance is capable of binding FcαR.

17. A method of delivering a substance to a mesenchymal cell comprising administering to an animal or cell in need thereof an effective amount of a conjugate comprising the substance coupled to an IgA receptor ligand.

18. A method according to claim 17 wherein the IgA receptor is pIgR or FcαR.

19. A method of detecting a condition associated with the activation of an IgA receptor on a mesenchymal cell comprising assaying a sample for (a) a nucleic acid molecule encoding an IgA receptor or a fragment thereof or (b) an IgA receptor or a fragment thereof.

20. A method according to claim 19 wherein the IgA receptor is pIgR or FcαR.

21. A method according to claim 19 or 20 wherein the condition is an inflammatory condition selected from arthritides, including rheumatoid arthritis, osteoarthritis, spondyloarthropathies, Crohn's disease, ulcerative colitis, Behcet's disease, Sjogren's disease and vasculitides.

ABSTRACT OF THE DISCLOSURE

A polymeric immunoglobulin receptor (pIgR) and FcαR have been found on synovial fibroblast cells and synovial tissues from patients with arthritis. Incubation of synovial cells with IgA causes proliferation of synovial cells. The invention relates to methods of modulating (preferably inhibiting) the proliferation of mesenchymal cells, methods of treating inflammatory conditions (such as arthritis), methods of modulating intracellular calcium signalling in mesenchymal cells. methods of drug delivery to mesenchymal cells and methods of detecting conditions associated with IgA receptors on mesenchymal cells.

Figure 1 – Immunofluorescence staining for pIgR and FcαR in OA and RA synovial fibroblasts.

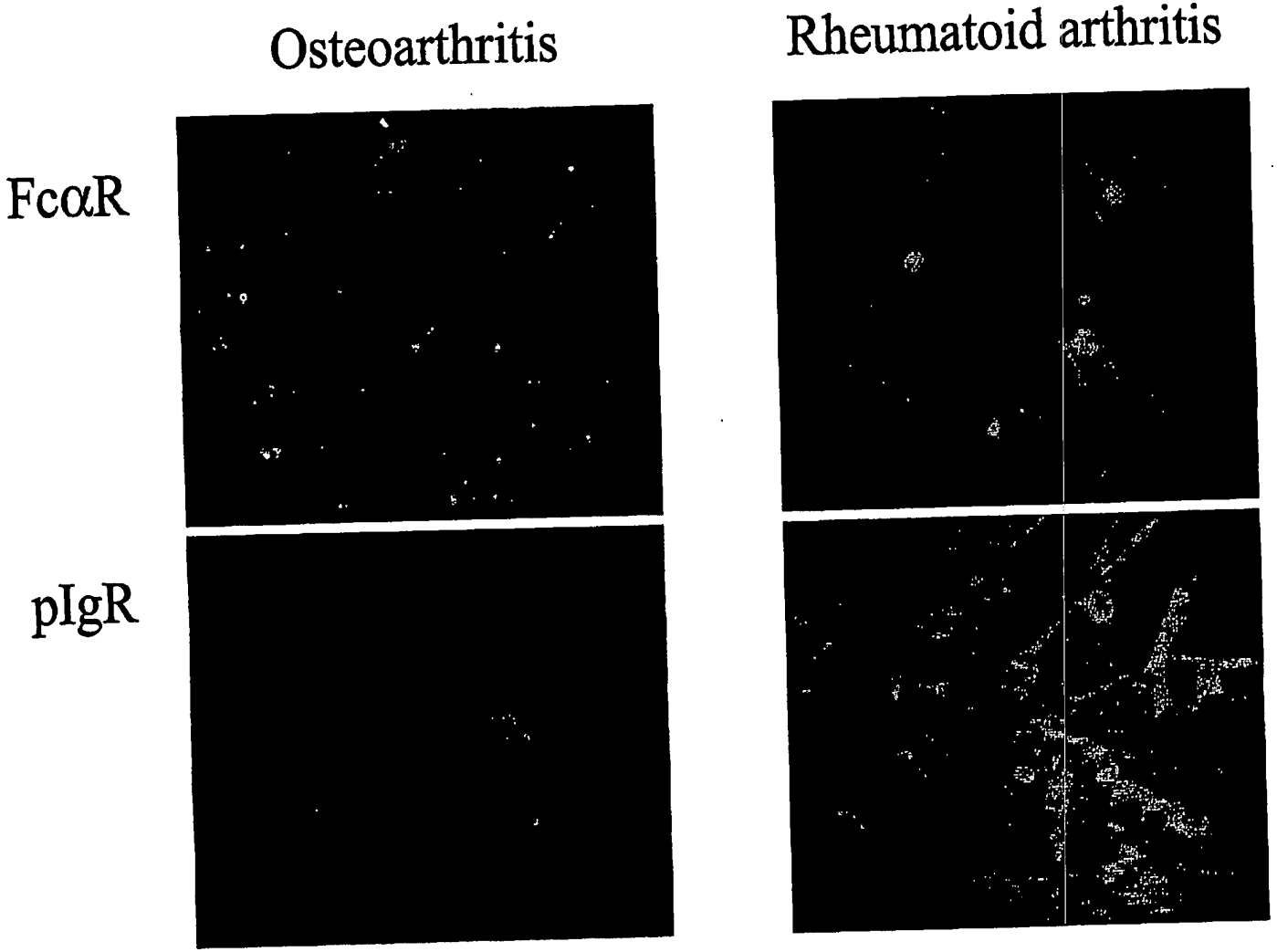


FIGURE 2 - RT-PCR for pIgR and FcαR

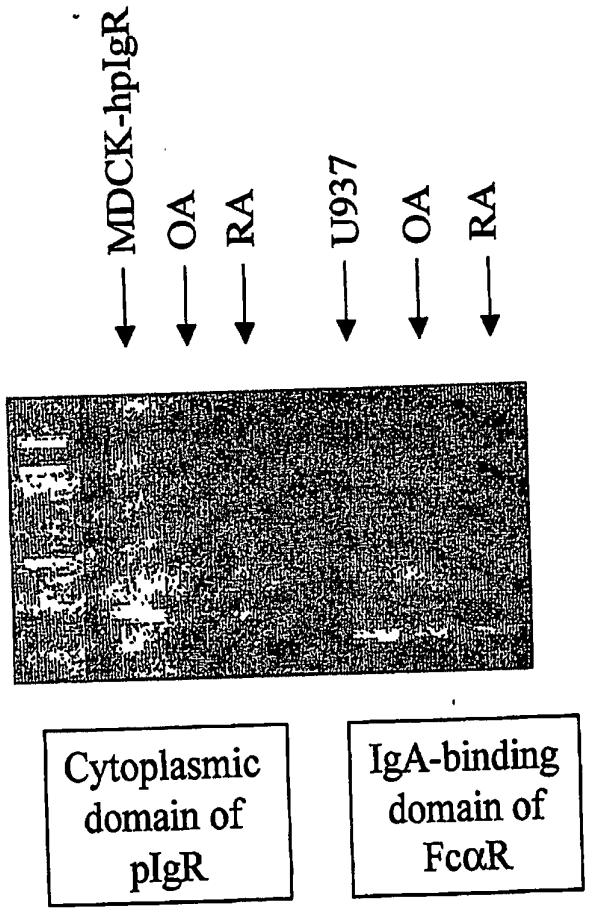
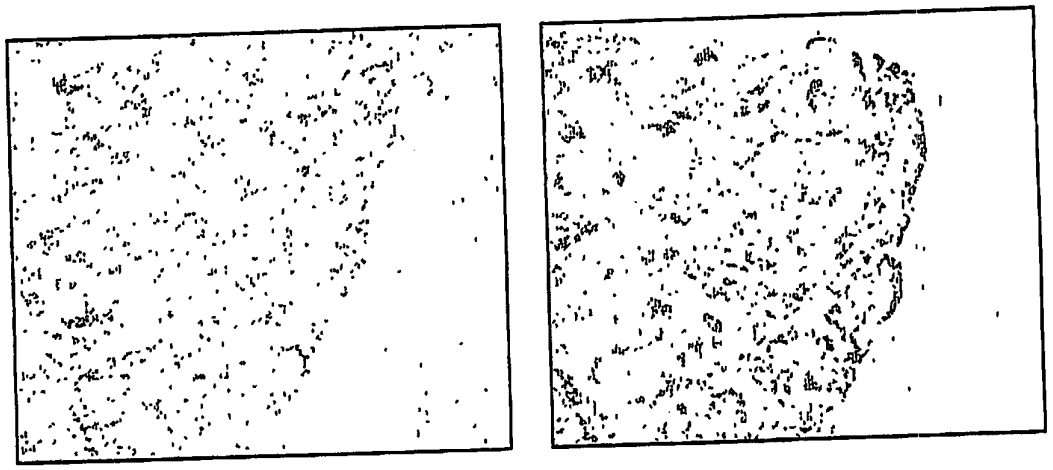
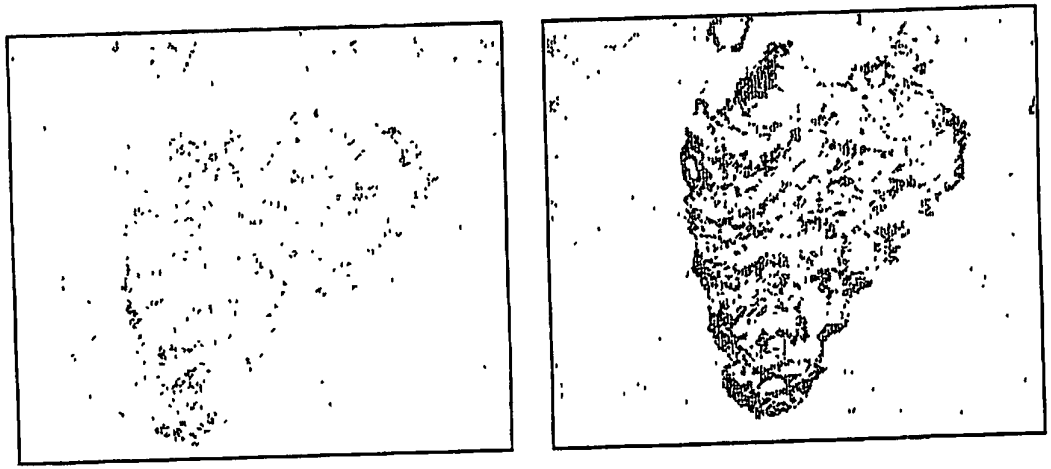


Figure 3 – Immunohistochemical staining for pIgR in synovial tissue from a patient with OA and RA

OA tissue:

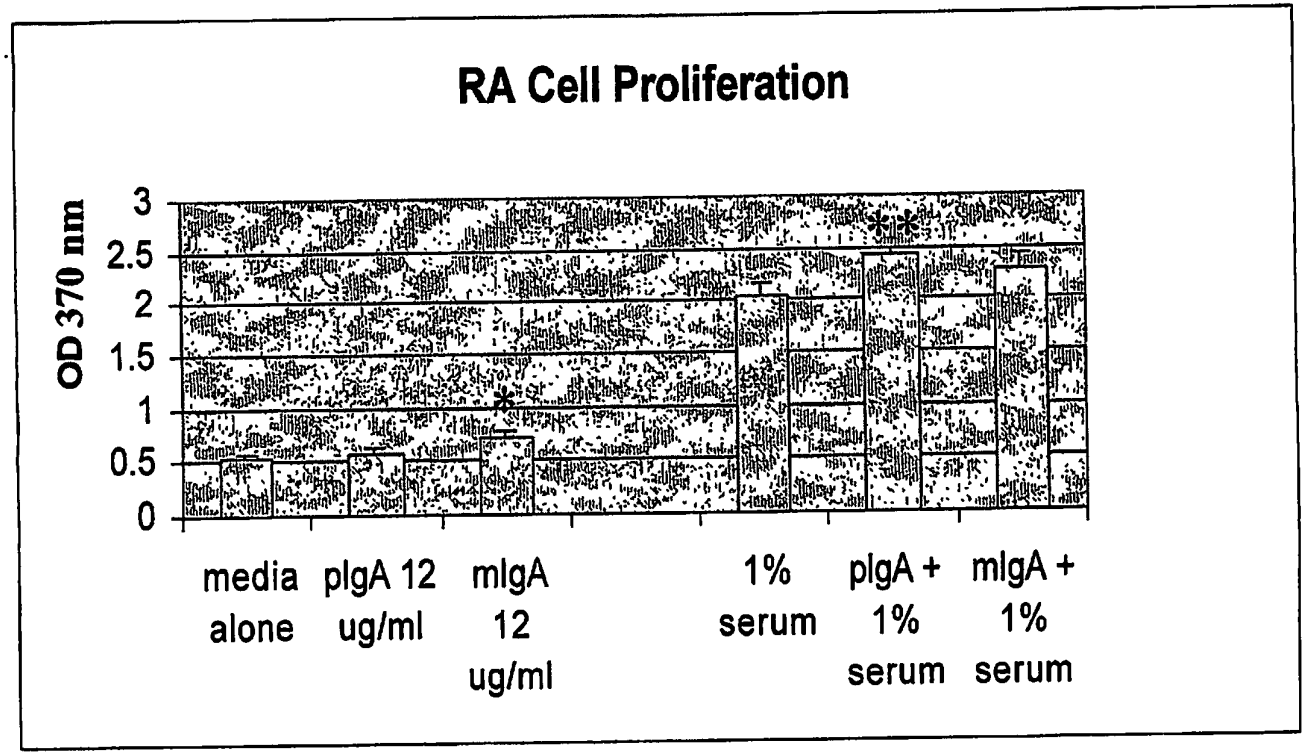


RA tissue:



HRP-conjugated Ab alone **Goat Ab to human SC**

Figure 4 – RA synovial cell proliferation



* Significantly increased compared to media alone.

** Significantly increased compared to 1% serum.

Figure 5 - pIgA Increases NFκB Activity in Synovial Fibroblasts in a Dose-Response Manner

Rheumatoid Arthritis **Osteoarthritis**

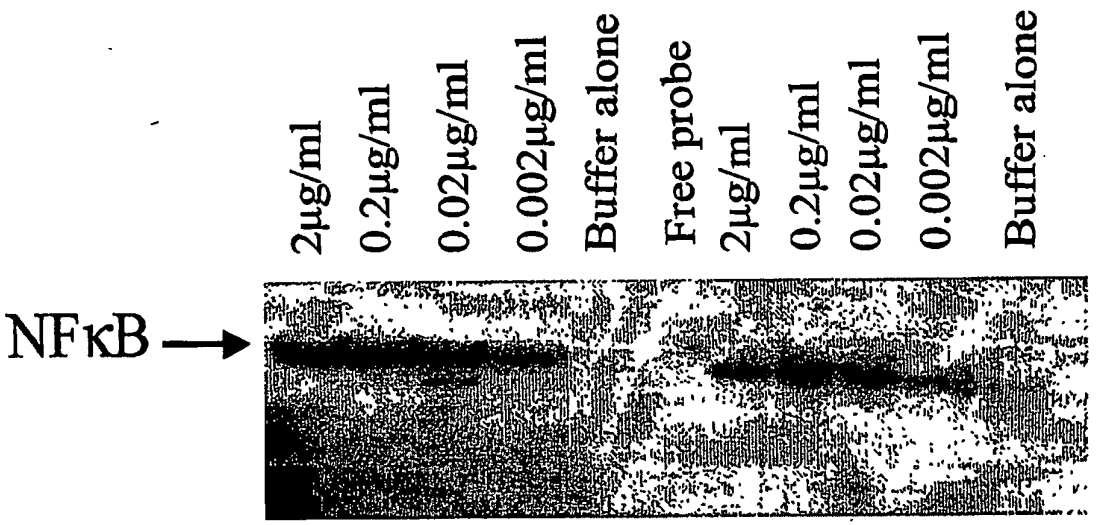
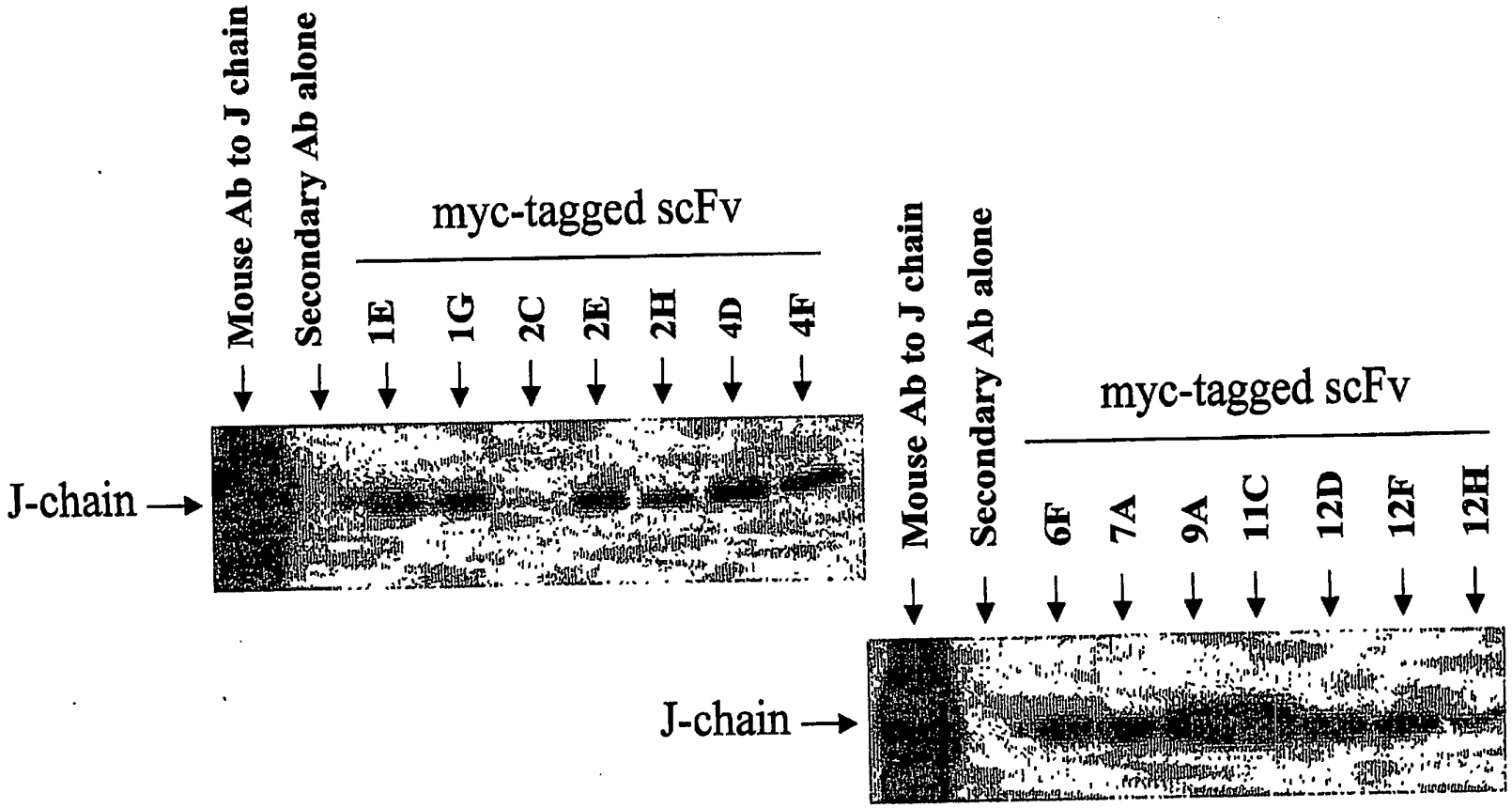


Figure 6: Western blots of candidate scFv binders to J-chain



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